Docket No.: BECTON 3.3-045
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Lynne Rainen

Application No.: 10/530,824 Confirmation No.: 7737

Filed: October 19, 2005 Art Unit: 1651

For: SAMPLE COLLECTION SYSTEM WITH CASPASE INHIBITOR Examiner: T. E. Underdahl

DECLARATION OF LYNNE RAINEN UNDER 37 CFR § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, LYNNE RAINEN, do declare as follows:

1. I have a Bachelor's Degree in Chemistry from the University of Missouri, and a Ph.D. in Biophysical Sciences from the University of Houston. I was also an NIH Post-Doctoral Fellow in the Department of Biochemistry, Tufts School of Medicine. I have been employed by Becton, Dickinson and Company, as a scientist for more than 13 years. My current title is Principal Scientist, Research and Development.

2. I am the only named inventor in this patent application. I have reviewed the U.S. Patent Office Communication mailed March 2, 2009, and publications discussed in it, including U.S. Patent 5,786,227, to David Edward Charlton, which I refer to as "*Charlton*", and Wilhelm, et al., Immunology Letters *59*:53-9 (1997), which I refer to as "*Wilhelm*". I disagree with the statement in the Communication that *Wilhelm* teaches that sodium azide is a caspase inhibitor.

3. In column 5, lines 11-12, *Charlton* states that sodium azide is a "preservative". *Wilhelm* reports that sodium azide, which had been previously reported to be an inducer of necrotic cell death, was capable of completely inhibiting apoptosis induced by VP-16/etoposide. VP-16 is known in the art as a topoisomerase inhibitor which blocks the reorganization of nuclear structure during cell division. *Wilhelm* concluded that sodium azide inhibited apoptosis induced by VP-16 at an early point in the apoptotic pathway, and ascribes the mechanism for this inhibition to the necrosis induction by sodium azide. The results of the experiments conducted by *Wilhelm* that concern caspase activity read as follows:

> 3.7 *Azide inhibits caspase-activation by VP-16*
>
> A family of cyseine proteases termed caspases [9] is thought to be essential for apoptosis and to become activated following an apoptotic signal [10]. A closely related subgroup of these caspases is able to cleae the cellular enzyme poly (ADP-ribose) polymerase after the peptide sequence DEVD [11] and such activity appears to be critical for apoptosis folling most if not all stimuli including etoposide [12,13]. We measured this activity as the ability to cleave the fluorogenic substrate DEVD-AMC (Fig. 6). ***Azide alone induced some DEVD-AMC-cleaving activity over a period of 8 h demonstrating further its ability to induce apoptosis.*** VP-16 caused strong activation of these proteases whereas azide completely inhibited the caspase-activating effect of VP-16 when both agents were present. (emphasis added)

As illustrated in the bar graph in Fig. 6, and as *Wilhelm* explicitly states in this quoted paragraph, sodium azide alone induced caspase activity. This is consistent with other reports in the literature that sodium azide mediates apoptosis by inducing caspase-3 cleavage. *See, e.g.*, Grammatopoulos, *et al.*,

Brain Res. Bull. *62(4)*:297-303 (2004), annexed to my Declaration as Exhibit A. On this basis alone, it is clear to me that sodium azide is not a caspase inhibitor. What *Wilhelm* does conclude is that sodium azide inhibited VP-16 induced apoptosis. In other words, sodium azide inhibits the activity of VP-16. Inhibiting a topoisomerase inhibitor with the result of arresting apoptosis simply cannot be equated with inhibition of caspases. All that can be drawn from *Wilhelm* is that sodium azide blocks the effect of a certain topoisomerase inhibitor. Thus, in my opinion, the conclusion stated in the Patent Office Communication regarding the activity of sodium azide is not at all supported by *Wilhelm*. If anything, *Wilhelm* reports to the contrary - that is, sodium azide induces caspase activity.

4. I declare under penalty of perjury that the foregoing is true and correct. I further state that I have been warned that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and such willful false statements may jeopardize the validity of the application or any patent resulting therefrom. I state that all statements made of my own knowledge are true and all statements made on information and belief, are believed to be true.

Executed on August 28, 2009

LYNNE RAINEN